The multiple roles of Orai N-terminus in CRAC channel function

Life Science Center
Johannes Kepler University

RBC, 2018
STIM1 and Orai - the main players in store-operated Ca^{2+} channels
Ca$^{2+}$ is a major factor regulating cellular processes

- proliferation
- muscle contraction
- cell growth
- exocytosis
- gene regulation
- apoptosis

major Ca$^{2+}$ entry pathway mediated by store-operated Ca$^{2+}$ channels
• SOC is dependent on the $[Ca^{2+}]$ in the stores
• store depletion triggers SOC activation

STIM1  Orai
**CRAC channel - STIM1 - Orai1**

- Ion pore at the center lined by six M1
- M1 and M4 helices ~20Å into the cytosol
- M4 extension of Orai1A/B pack into pairs involving L316 (L273) and L319 (L276)

Hou et al., Science 2012

Isabella Derler
University of Linz, Austria
• Introduction
• Techniques
  • Patch Clamp Technique
  • Fluorescence Resonance Energy Transfer (FRET) Microscopy
• Results
• Summary
Activation of store-operated Orai1 channels

STIM1 and Orai1 over-expression in HEK cells

-> Store depletion in HEK cells co-expressing STIM1 and Orai1 activates store-operated Ca\(^{2+}\) currents
STIM1 and Orai1 required for channel activity

-> Co-expression of STIM1 and Orai1 activates store-operated Ca\(^{2+}\) currents
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Förster Resonance Energy Transfer (FRET)

- FRET is the radiationless transfer of emission energy from a donor to an acceptor that occurs beyond a distance of 10 nm.

**Nice technique to detect protein-protein interactions**

- Cyan and yellow fluorophores are used from *Aequorea victoria* – keeper of GFP

- A pixel per pixel readout allows a time-dependent measurement of FRET within a single cell

Isabella Derler
University of Linz, Austria
Direct coupling of STIM1 to Orai1 results in Ca$^{2+}$ channel activation.
STIM1 oligomerisation

Isabella Derler
University of Linz, Austria
STIM1 forms stable homomers after store store depletion with Thapsigargin (TG)

Muik et al., JBC 2008
STIM1 to Orai1 coupling

Muik et al., JBC 2008
STIM1 to ORAI1 coupling is temporally as well as spatially correlated with an increase in FRET

Muik et al., JBC 2008
STIM1 C-terminus is sufficient for ORAI1 activation

Muik et al., JBC 2008
STIM1-mediated ORAI1 conformational change

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University of Linz, Austria
STIM1-mediated ORAI1 conformational change

Global conformational change of Orai channel upon activation

V102A G98A H134A L185A A137V V181A W176C L138A P245L


Isabella Derler
University of Linz, Austria
1) Activation of STIM1

2) Coupling of STIM1-Orai1

3) Orai1 pore opening
STIM1 adjusts authentic CRAC channel hallmarks

Derler et al., JBC; 2018
Orai1 C-terminus indispensable for binding to STIM1

Muik et al., JBC; 2008
Orai1 N-terminus essential for STIM1-mediated Orai1 gating

Muik et al., JBC; 2008
Last 15aa of Orai1 N-terminus are indispensable for STIM1 mediated Orai1 gating
Last 15aa of Orai1 N-terminus are indispensable for STIM1 mediated Orai1 gating

ETON region

TM1 domain

Store-operated activation

Derler et al., JBC; 2013
Orai1 N-terminus mediates authentic CRAC channel characteristics

Store-operated activation
CRAC channel hallmarks

Derler et al., JBC; 2018
Isoform specific Orai channel activation

Bergsmann et al., JBC, 2011; Fahrner et al., JBC, 2018

ORAI1  68 EHS MQALSWRKLY LSRAKLKASS RTSALLSGFA MVAMVEVQLD
ORAI3  43 QHS LRALSWRRLY LSRAKLKASS RTSALLSGFA MVAMVEVQLE

Store-operated activation

Isabella Derler
University of Linz, Austria
Orai1 N-terminus communicates with loop2 to mediate Orai channel gating

Orai1 ΔN1-78
Orai3-L2

Orai3 ΔN1-53
Orai1-L2

Fahrner et al., JBC; 2018
Orai1 N-terminus communicates with loop2 to mediate Orai channel gating

Fahrner et al., JBC; 2018

Isabella Derler
University of Linz, Austria
Orai1 N-terminus communicates with loop2 to mediate Orai channel gating

Fahrner et al., JBC; 2018

Isabella Derler
University of Linz, Austria
Gating of Orai1 is further controlled via a communication of N-terminus with cholesterol

(L/V)-X_{1-5}-(Y)-X_{1-5}-(K/R) – consensus motif of cholesterol binding motif

Derler et al., Sci Signal; 2016
Gating of Orai1 is further controlled via a communication of N-terminus with cholesterol

(L/V)-X$_{1-5}$-(Y)-X$_{1-5}$-(K/R) – consenus motif of cholesterol binding motif

Derler et al., Sci Signal; 2016
Gating of Orai1 is further controlled via a communication of N-terminus with cholesterol

Derler et al., Sci Signal; 2016

Isabella Derler
University of Linz, Austria
Cholesterol binding is affected by a mutated Orai1 N-terminus

Derler et al., Sci Signal; 2016
1. Upon STIM1 multimerisation, it couples directly to Orai1 via coiled-coil domains in punctae structures.

2. Binding results in Calcium influx via Orai1 channels

3. Orai1 C-terminus is main binding site for STIM1

4. Orai1 N-terminus:
   - Control STIM1 coupling partly
   - Essential for store-operated activation
   - Controls CRAC channel hallmarks
   - Controls distance to Orai1 loop2
   - Is involved in cholesterol mediated Orai regulation.
Several hot spots within Orai1 N-terminus control Orai1 gating

Isabella Derler
University of Linz, Austria

Derler et al., JBC; 2013
STIM1 to Orai coupling via coiled-coil domains

Orai 1

Orai 2

Orai 3

L273S

L373S
STIM1 to Orai coupling via coiled-coil domains

Isabella Derler
University of Linz, Austria
STIM1 to Orai coupling via coiled-coil domains

Frischauf et al., JBC 2009
STIM1 to Orai coupling via coiled-coil domains

**CFP-Orai1 + STIM Cterminus-YFP**

- FRISHAUF et al., JBC 2009

**CFP-Orai1_L273S+STIM_Cterminus_YFP**

**CFP-Orai1 + STIM_Cterminus_L373S-YFP**

Isabella Derler
University of Linz, Austria
Regulation of STIM1 C-terminal coupling to Orai

Which domains within STIM1 C-terminus are required to activate Orai channels?
Conformational rearrangement of STIM1 C-terminus

Muik et al., EMBO J 2011

Isabella Derler
University of Linz, Austria
Double labeled STIM1 C-terminus is functional

Double labeled STIM1 C-terminus constitutively activates Orai1 currents